Temperature-sensitive DNA aptamer blocking enzymatic activity of Twa DNA polymerase

Polymerase chain reaction (PCR) is a basic method in many fields of molecular biology and diagnosis, as well as an important tool for gene cloning and analysis. Taq DNA polymerase isolated from *Thermus aquatics* is the most widely used enzyme for PCR-based diagnosis. However, the enzyme does not have proofreading activity, and therefore is not suitable for gene cloning and amplification of long DNA fragments. To solve this problem, we recently isolated from *Thermococcus waiotapuensis* the enzyme Twa DNA polymerase, which has 3'-5' exonuclease-dependent proofreading activity and is able to amplify longer DNA fragments than the Taq DNA polymerase. Although temperature-resistant DNA polymerases have maximum enzymatic activity at about 70°C, some polymerase activity is also observed at room temperature. Because at this temperature oligonucleotide primers could bind to the DNA template nonspecifically, unwanted nonspecific fragments are often generated when PCR is set up at room temperature. To prevent generation of the nonspecific fragments, hot-start PCR was developed in which the enzyme activity of DNA polymerase at temperature <40°C is blocked by chemical modification or by antibodies specific for the active site of the enzyme. Higher temperature attained during the first denaturation step of PCR (usually about 94°C) removes the inhibitory effect of the inhibitors. These strategies for hot-start PCR have drawbacks, because they reduce activity of the enzyme and/or are irreversible. An alternative method for hot-start PCR is to use a DNA aptamer which, similarly to antibodies, binds to the enzyme and inhibits its activity at low temperature. In contrast to antibodies and chemical modifications, inhibition of enzymatic activity by DNA aptamers is reversible and usually without any effect on the enzymatic activity of polymerases. However, there are no DNA aptamers capable of inhibiting Twa DNA polymerase activity.

In this project we used modified SELEX (Systematic Evolution of Ligands by Exponential Enrichment) and found a sequence of a DNA aptamer that is capable of inhibiting the enzymatic activity of Twa DNA polymerase at a temperature below 50°C (Figure 1).

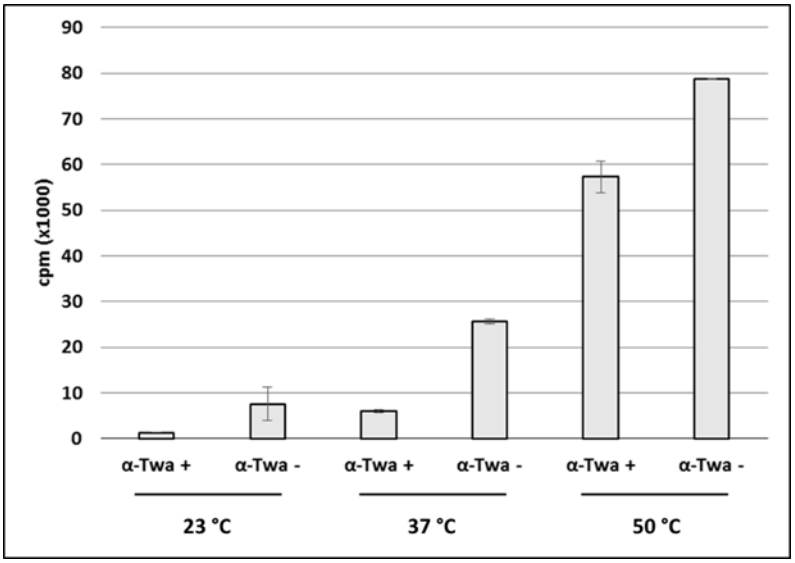

Figure 1. Inhibitory effect of the DNA aptamer on the enzymatic activity of Twa DNA polymerase at different temperatures. Twa DNA polymerase activity was determined in an assay in which ^{32}P-ATP incorporation into genomic salmon testes DNA in the presence of 0.2 mM dNTPs and 0.05 U Twa DNA polymerase in Twa DNA polymerase reaction buffer was measured after 30 min incubation at different temperatures with (α-Twa +) or without (α-Twa -) anti-Twa DNA aptamer.

Further studies showed that anti-Twa DNA aptamer at concentrations 3 - 12 nM prevented formation of non-specific DNA amplicons when the PCR mixture was incubated before PCR for 30 min at 23°C (Figure 2).

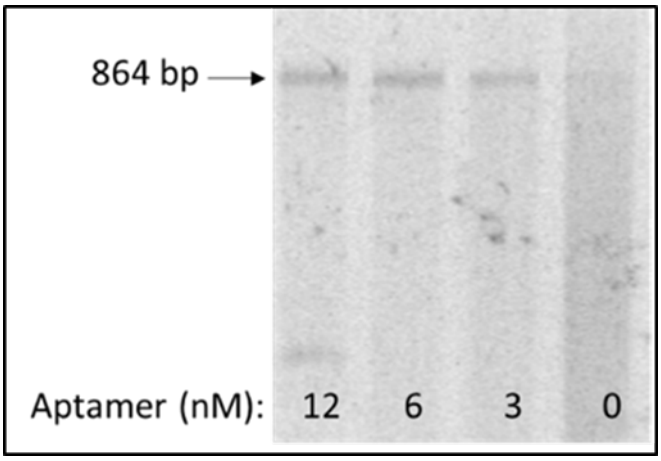

Figure 2. Effect of the anti-Twa DNA aptamer at different concentrations on PCR with Twa DNA polymerase. Mouse genomic DNA fragment (864 bp) was amplified in PCR with Twa DNA polymerase without or with various concentrations of anti-Twa DNA aptamer. For PCR, the reaction mixture (0.2 mM dNTP, 1 U Twa DNA polymerase, 0.5 µM forward and reverse oligonucleotide primers, and Twa polymerase buffer) was prepared and incubated at 23°C for 30 minutes before transfer to T100 thermal cycler (BioRad). The following cycling conditions were used: initial denaturation at 94 ˚C for 1 min followed by 40 cycles of denaturation at 95 ˚C for 15 sec, annealing at 55 ˚C for 15 sec, and elongation at 72 ˚C for 45 sec. PCR amplicons were fractionated by electrophoresis in 1% agarose gel and stained with ethidium bromide. Specific DNA amplicons (864 bp; position indicated by an arrow) were produced in PCR mixtures supplemented with anti-Twa DNA aptamer at a concentration 3 – 12 nM.

Conclusion: The results show that the anti-Twa DNA aptamer inhibits Twa DNA polymerase activity and is suitable for hot-start PCRs that use Twa DNA polymerase.

To get more information on this project and/or to purchase a nonexclusive license for obtaining the anti-Twa inhibitory aptamer, please, contact the Centre for Technology Transfer, IMG AS CR, Videnska 1083, 14220 Prague 4, Czech Republic; Tel. (420-241 063 227 or 420-602 892 876).